

FLAVONOIDS OF THE PROTEACEAE, PART 1. A CHEMICAL CONTRIBUTION TO STUDIES ON THE EVOLUTIONARY RELATIONSHIPS IN THE S. AFRICAN PROTEOIDEAE*

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ABSTRACT

The leaves of 103 species of Proteoideae were hydrolysed with hot mineral acid and the hydrolysates were examined by paper chromatography for the presence of chemotaxonomically significant flavonoids. On the hypothesis that the presence of myricetin and leucodelphinidin indicate primitive characters whereas their absence more advanced characters, a scheme representing possible evolutionary relationships within the Proteoideae has been tentatively advanced. The results of this survey also indicate that in the biosynthesis of myricetin in the leaf, leucodelphinidin may be a precursor.

UITTREKSEL

FLAVONOIDE VAN DIE PROTEACEAE, DEEL 1. 'N CHEMIESE BYDRA TOT DIE STUDIES VAN DIE ONTWIKKELINGS VERWANTSAPPE IN DIE SUID-AFRIKAANSE PROTEOIDEAE. Die blare van 103 Proteoideae spesies was gehidroliseer met warm mineraal suur en die hidrolisate was ondersoek met papier chromatografie vir die teenwoordigheid van chemotaksonomies belangrike flavonoide. Op die hipotese dat die teenwoordigheid van myricetin en leucodelphinidien, op primitiewe eienskappe aandui en hul afwesigheid, op meer gevorderde eienskappe, 'n raamwerk wat beweer dat daar moontlike evolutionêre verbandskappe in die Proteoideae is word voorlopig geopper. Die resultaat van hierdie ondersoek dui ook daarop aan dat leucodelphinidien die voorloper van die biosintese van myricetin in 'n blaar, mag wees.

INTRODUCTION

The family Proteaceae comprising over 1000 species is essentially peculiar to the southern continents (Hutchinson 1959). On morphological grounds it has been subdivided into two sub-families:

- (i) the Proteoideae, to which most of the species indigenous to Southern Africa belong, and
- (ii) the Grevilleoideae into which most of the Australian species fall.

This paper is concerned with the former subfamily, many of which are to be found in the Western Cape region. It was considered that a study of the flavonoid constituents of the Proteaceae could prove fruitful because the bark of many species was used for tanning purposes during the early Cape days

(*This paper was presented in part at a meeting of the Experimental Biology Group, Mowbray, Cape, on 31st October, 1970.)

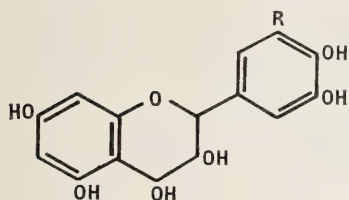
Accepted for publication 16th March, 1971.

(Watt and Breyer-Brandwijk, 1962). Reviews on the flavonoid chemistry of the Proteaceae by Watt and Breyer-Brandwijk and by Hegnauer (1969) indicate that the coverage to date has been superficial. The most important contribution is the chemotaxonomic survey of eight species by Bate-Smith (1962) and a further thirty species by Van Oudtshoorn (1963). These workers subjected leaves of selected species to hot acid hydrolysis and then used paper chromatography to identify flavonoids and other plant phenolics in each resulting hydrolysate.

The usefulness of flavonoids as taxonomic guides has been discussed by Bate-Smith (1963). The results of his survey on the phenolic constituents in eight hundred species of dicotyledons (Bate-Smith 1962) following on an earlier study (Bate-Smith 1957) led Bate-Smith to conclude that the presence of leucoanthocyanins, as indicated by the production of red colours upon acid hydrolysis, and the presence of compounds having three vicinal hydroxy groups on a benzene ring as found, for example, in leucodelphinidin **2** and myricetin **5**, are both primitive characters, and that the loss of the genes responsible for the biosynthesis of these compounds in the evolutionary line is apparently an irreversible process (Bate-Smith 1962).

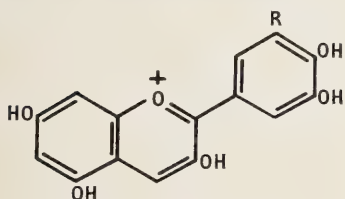
It would appear that Bate-Smith's deductions could be applied to determining evolutionary trends within a given plant family and so complement the work of morphologists and geneticists. A scheme representing the possible evolution of the Proteoideae has for example been advanced by Johnson and Briggs (1963), based upon chromosomal studies. We felt that an extension of the survey of Van Oudtshoorn to include as many of the local Proteaceae as possible might shed additional light on this subject. Van Oudtshoorn himself, in observing myricetin to be present only in the genera *Leucospermum* and *Leucadendron* but not in *Aulax*, *Mimetes*, *Paranomus* and *Protea*, has already commented on the potential of this flavonol for infrafamilial taxonomic purposes. It was therefore decided to examine other species within the genera already examined as well as other genera such as *Diastella*, *Serruria*, *Sorocephalus* and *Spatalla* which have not been studied. It was further decided at this stage to restrict the study to the determination of the presence or absence of the chemotaxonomically significant flavonoids, viz. the leucocompounds leucocyanidin **1** and leucodelphinidin **2**, and the flavonols kaempferol **3**, quercetin **4** and myricetin **5**.

The presence of the leuco-compounds **1** and **2** is shown by the appearance of their respective pigments cyanidin **6** and delphinidin **7**, both formed as a result of oxidation of the leuco-compounds on heating in the presence of mineral acid (Bate-Smith 1962). This same treatment hydrolyses the glycosides of the flavonols **3**, **4** and **5** if these be present in the original material.



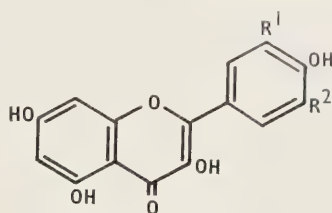
1 (R = H), LEUCOCYANIDIN

2 (R = OH), LEUCODELPHINIDIN



6 (R = H), CYANIDIN

7 (R = OH), DELPHINIDIN



3 (R¹ = R² = H), KAEMPFEROL

4 (R¹ = H; R² = OH), QUERCETIN

5 (R¹ = R² = OH), MYRICETIN

METHODS AND RESULTS

Except where indicated, leaves were collected from labelled plants growing in the National Botanic Gardens, Kirstenbosch, Cape. The freshly-picked leaves were dried in an air oven at 60°C for 24 hours. Chlorophyll and waxes were removed first by subjecting a macerated sample (*ca.* 2 g) for 1–3 hour extraction by light boiling petroleum (b.p. 40–60°C) in a Soxhlet extractor. The sample was then covered with 3 *M* hydrochloric acid (20 ml) in a boiling tube which was immersed in a boiling water bath for 20 to 30 minutes. The hydrolysate was cooled, extracted first with diethyl ether (AnalaR grade, 3×15 ml) to extract the flavones and other phenolics, then with 1-pentanol (2–3 ml) to extract the anthocyanidins. The ethereal extract was washed with water (3 ml) to remove residual acid, then dried over anhydrous sodium sulphate (5 g). The filtered extract was then concentrated to low volume (*ca.* 0.5 ml) for chromatographic examination.

The ether extracts were spotted onto strips of previously washed Whatman paper No. 1, together with myricetin and quercetin as markers. One strip was developed in the Forestal solvent (acetic acid- concentrated hydrochloric acid-water, 30:3:10) and the other in 60% acetic acid. Descending chromatography was used in all cases. When the solvent had moved 25 cm the chromatograms were dried and examined under ultraviolet light before and after exposure to ammonia vapour. Except where “streaking” occurred, myricetin and quercetin

were easily identified. Kaempferol was identified by its R_f values, 0.56 (Forestal) and 0.46 (60% acetic acid), and its yellow fluorescence under UV light. It was further confirmed by applying the extract to a strip of Whatman No. 1 paper as a band. After development in 60% acetic acid, the band at R_f 0.46 was cut out, extracted into spectroscopically pure ethanol and the spectrum was recorded on a self-recording Beckmann DB spectrophotometer. The absorption band at 368 nm was shifted to 426 nm on addition of aluminium chloride while anhydrous sodium acetate shifted the band at 268 to 275 nm (Jurd, 1962). Other flavonoids and phenolics which were observed on the chromatograms are not reported in this survey.

In a few cases where streaking tended to mask the presence of the flavonols, thin layer chromatography was applied. The ether extracts were spotted onto aluminium sheets coated with polyamide F₂₅₄ (Merck precoated) together with the marker flavonols, and the plates were developed in a methanol-acetic acid-water solvent (90:5:5) (Mabry, Markham and Thomas, 1970). After drying the plates were examined under both 240 and 340 nm lights.

The colour of the 1-pentanol extract with the pigments varied from pink to red-brown. The presence or absence of leucoanthocyanins was estimated visually from the intensity of the colour of this extract. The extracts were then applied to previously washed Whatman No. 1 filter paper strips and, together with cyanidin and delphinidin as markers, using descending technique, one strip was developed in the Forestal solvent (above) and the other in the "Roux" solvent, 90% formic acid- 3 M hydrochloric acid (1:1) (Roux, 1957). The chromatograms were dried when the solvent front had moved 25 cm and were examined under UV light. With the prior removal of flavones and other phenolic compounds in the ether extract, both cyanidin and delphinidin were readily identified except where streaking occurred. When these pigments occurred together in the same hydrolysate their relative proportions were estimated approximately by visual comparison of the intensities of the spots on the dry paper chromatograms.

The detailed results of this study on one hundred and three species are reported in Table I.

Since Van Oudtshoorn (1963) had worked on preserved herbarium specimens, we decided to re-examine those species which were available in the Gardens and which he had examined. The names of several species had changed since his study and more especially we wished to assess the relative proportions of cyanidin and delphinidin in the hydrolysates. Where our results appeared to differ from those of Van Oudtshoorn, we have reported our own. Thus Van Oudtshoorn reported myricetin to be present in *Leucadendron ramosissimum* whereas we found this flavonol to be absent from *L. conicum* (= *L. ramosissimum*). We also report the presence of both myricetin and leucodelphinidin in *L. galpinii* whereas

TABLE I
Distribution of Chemotaxonically-significant Flavonoids among some South African Proteoideae^a

Species ^b	L-A	D	C	M	Q	K
<i>Aulax cancellata</i> (L.) Druce (= <i>A. pinifolia</i> Berg.)	+++	++	(+)	—	+	+
<i>A. pallasia</i> Stapf ^c	+++	++	(+)	?	(+)	?
<i>A. umbellata</i> (Thunb.) R.Br. (= <i>A. cneorifolia</i> Salisb. ex Knight ^e)	++	+	(+)	—	++	++
<i>Brabeium stellatifolium</i> L. ^d	—	—	—	—	—	—
<i>Diastella bryiflora</i> Salisb. ex Knight ^e	+++	?	+	?	+	?
<i>Faurea macnaughtonii</i> Phill.	+	?	?	—	(+)	?
<i>Leucadendron album</i> (Thunb.) Fourcade	+++	—	+	—	+	(+)
<i>L. argenteum</i> (L.) R. Br.	++	+	—	—	+	(+)
<i>L. conicum</i> (Lam.) Williams (= <i>L. ramosissimum</i> Buck and Meisn.) ^e	+++	++	(+)	?	++	(+)
<i>L. cryptocephalum</i> Guthrie	+++	++	(+)	++	++	++
<i>L. daphnoides</i> (Thunb.) Meisn.	+++	—	+	—	++	++
<i>L. discolor</i> Buell ex Phill. and Hutch.	+++	++	(+)	(+)	+	+
<i>L. galpinii</i> Phill. and Hutch. ^e	+++	++	++	+	+	?
<i>L. gandogerii</i> Schinz ex Gandoger (= <i>L. guthrieae</i> Salter)	+++	++	(+)	++	+	++
<i>L. globularia</i> (Lam.) Williams	++	++	+	?	(+)	(+)
<i>L. lanigerum</i> Meisn.	+++	++	(+)	—	+	+
<i>L. lauroleum</i> (Lam.) Williams	+++	+	(+)	+	+	+
<i>L. longmorensis</i> Williams	+++	++	(+)	—	+	++
<i>L. loranthifolium</i> (Salisb. ex Knight) Williams	+++	—	+	—	(+)	++
<i>L. meyerianum</i> Buell ex Phill. and Hutch.	+++	—	++	—	+	++
<i>L. modestum</i> Williams	+++	++	+	—	+	+
<i>L. muirii</i> Phill.	+++	?	++	—	++	—
<i>L. nobile</i> Williams	++	—	++	—	++	+
<i>L. rubrum</i> Burm. f.	+++	++	(+)	+	++	+
<i>L. sabulosum</i> Salter	+++	++	(+)	—	?	+
<i>L. salicifolium</i> (Salisb.) Williams	+++	++	(+)	(+)	++	+
<i>L. salignum</i> Berg. (= <i>L. adscendens</i> R. Br.) ^e	+++	++	(+)	+	++	++
<i>L. sessile</i> R. Br.	+++	?	++	++	++	?
<i>L. stellare</i> Sims	+++	++	(+)	+	+	++
<i>L. tinctum</i> Williams	+++	?	+	++	++	?
<i>L. tortum</i> R. Br.	+++	+	+	?	+	++
<i>L. uliginosum</i> R. Br.	+++	++	+	+	++	?
<i>L. xanthoconus</i> (O. Kuntze) K. Schum. (= <i>L. salignum</i> R. Br.) ^e	+++	++	(+)	++	++	?
<i>Leucospermum bolusii</i> Gandoger	+++	++	(+)	+	++	++
<i>L. catherinae</i> Compton	+++	++	(+)	(+)	+	++
<i>L. conocarpodendron</i> (L.) Buell	+++	+	—	—	?	+
<i>L. cordifolium</i> (Salisb. ex Knight) Fourcade	+++	+	—	+	+	+
<i>L. cuneiforme</i> (Burm. f.) Rourke (= <i>L. attenuatum</i> R. Br.)	+++	++	(+)	?	++	++
<i>L. lineare</i> R. Br. ^e	+++	++	(+)	?	+	++
<i>Leucospermum muirii</i> Phill.	+++	++	+	?	++	++
<i>L. patersonii</i> Phill.	+++	++	(+)	+	+	(+)
<i>L. prostratum</i> (Thunb.) Stapf ^c	+++	++	+	+	+	+
<i>L. reflexum</i> Buell ex Meisn.	+++	++	(+)	+	+	+

TABLE I (continued)

Distribution of Chemotaxonomically-significant Flavonoids among some South African Proteoideae^a

Species ^b	L-A	D	C	M	Q	K
<i>L. rodolentum</i> (Salisb. ex Knight)						
Rourke	+++	++	(+)	++	+	+
<i>L. tottum</i> (L.) R. Br.	+++	++	(+)	?	(+)	+
<i>L. truncatum</i> (Salisb. ex Knight)						
Rourke	++	++	(+)	+	+	(+)
<i>L. vestitum</i> (Lam.) Rourke	+++	++	(+)	?	+	++
<i>Mimetes cucullatus</i> (L.) R. Br. (= <i>M. lyrigera</i> Salisb. ex Knight ^c)	+++	—	++	—	+	(+)
<i>M. fimbriaefolius</i> Salisb. ex Knight	+++	—	(+)	—	+	—
<i>M. hottentoticus</i> Phill. ^c	+++	—	+	—	+	+
<i>Orothamnus zeyheri</i> Hook. f. [†]	+++	—	++	+	+	?
<i>Paranomus reflexus</i> (Phill. and Hutch.) N. E. Br.	+++	+	(+)	—	(+)	+
<i>P. spicatus</i> (Berg.) R. Br. ^c	++	(+)	—	?	+	++
<i>Protea acaulis</i> (L.) Reich	++	?	?	?	?	?
<i>P. acerosa</i> R. Br.	+	?	?	?	?	?
<i>P. amplexicaulis</i> (Salisb.) R. Br.	++	?	?	?	+	?
<i>P. angustata</i> R. Br.	+	?	?	—	?	—
<i>P. arborea</i> Houtt. (= <i>P. grandiflora</i> Thunb.)	++	—	—	—	?	?
<i>P. aristata</i> Phill.	—	?	?	?	?	?
<i>P. barbigera</i> Meisn.	++	—	(+)	—	—	(+)
<i>Protea canaliculata</i> Haw	++	—	?	—	(+)	?
<i>P. cedromontana</i> Schlechter	++	—	—	—	++	++
<i>P. compacta</i> R. Br.	++	?	(+)	—	++	?
<i>P. cordata</i> Thunb.	++	—	—	?	?	?
<i>P. cynaroides</i> (L.) L. ^g	+	—	—	—	(+)	—
<i>P. decurrens</i> Phill.	++	—	—	—	+	+
<i>P. eximia</i> (Salisb. ex Knight) Fourcade	+	—	—	?	(+)	?
<i>P. glabra</i> Thunb.	+	?	?	?	?	?
<i>P. grandiceps</i> Tratt.	—	?	?	?	?	?
<i>P. harmeri</i> Phill.	+++	—	++	—	++	++
<i>P. lanceolata</i> E. Mey. ex Meisn.	(+)	—	—	—	?	?
<i>P. laurifolia</i> Thunb. ^c (= <i>P. marginata</i> Thunb. ^c)	+++	?	+	—	++	?
<i>P. longiflora</i> Lam.	+	?	?	?	?	?
<i>P. longifolia</i> Andr.	—	?	?	?	?	+
<i>P. lorifolia</i> (Salisb. ex Knight) Fourcade	++	—	+	—	++	(+)
<i>P. macrocephala</i> Thunb. (= <i>P. incompta</i> R. Br. ^c)	++	?	?	—	+	?
<i>P. minor</i> Compton	+	?	?	—	?	+
<i>P. mundii</i> Klotzsch ^c	+	?	?	—	?	?
<i>P. nana</i> (Berg.) Thunb.	++	?	?	—	+	?
<i>P. neriifolia</i> R. Br. ^c	+	—	(+)	—	?	+
<i>P. obtusifolia</i> Buek ex Meisn.	+	?	?	—	—	—
<i>Protea odorata</i> Thunb.	++	?	?	—	+	+
<i>P. pityphylla</i> Phill.	+++	—	(+)	—	+	—
<i>P. pulchra</i> Rycroft	++	—	—	—	++	+
<i>P. repens</i> L. (= <i>P. mellifera</i> Thunb.)	—	—	—	—	+	?
<i>P. restionifolia</i> (Salisb. ex Knight) Rycroft	+++	—	+	—	+	+
<i>P. roupelliae</i> Meisn.	+++	—	++	—	+	(+)
<i>P. rubropilosa</i> Beard	++	—	—	—	?	—

TABLE I (continued)

Distribution of Chemotaxonomically Significant Flavonoids among some South African Proteoideae^a

Species ^b	L-A	D	C	M	Q	K
<i>P. rupicola</i> Meisn.	+	—	—	—	?	?
<i>P. scabra</i> R. Br.	+++	—	(+)	?	+	?
<i>P. scolymocephala</i> (L.) Reich.	+	?	?	?	?	?
<i>P. simplex</i> Phill. ^{c,h}	++	?	+	?	(+)	?
<i>P. speciosa</i> (L.) L.	++	?	+	—	+	(+)
<i>P. stokoei</i> Phill.	+	?	?	—	?	?
<i>P. sulphurea</i> Phill.	++	?	?	—	(+)	—
<i>P. venusta</i> Compton	++	?	?	—	+	(+)
<i>P. welwitschii</i> Engl. ⁱ (= <i>P. hirta</i> Klotzsch ^g)	++++	++	+	—	+	(+)
<i>P. witzbergiana</i> Phill.	++	?	+	—	+	(+)
<i>Serruria ascendens</i> R. Br.	+++	+	+	++	++	++
<i>S. aemula</i> R. Br.	++	++	+	++	?	?
<i>S. barbigeria</i> Salisb. ex Knight	+++	++	+	+	+	(+)
<i>S. florida</i> (Thunb.) Salisb. ex Knight	++	++	(+)	++	++	?
<i>Serruria pedunculata</i> (Lam.) R. Br. (= <i>S. artemesiaefolia</i> Salisb. ex Knight)	+++	++	+	?	+	+
<i>Sorocephalus lanatus</i> (Thunb.) R. Br. ^j	+++	++	(+)	?	+	+
<i>Spatalla curvifolia</i> Salisb. ex Knight.	++	+	+	—	+	+
<i>S. incurva</i> (Thunb.) R. Br. ^k	+++	++	(+)	+	+	?

Footnotes to Table I.

^a The abbreviations are as follows: L-A = leucoanthocyanin reaction, with D and C = delphinidin and cyanidin respectively, each formed from a leucocompound; M = myricetin; Q = quercetin; K = kaempferol. The symbols +++, ++, +, (+) and — refer to the relative strength or absence of the particular constituent in question. Where there is doubt about the presence of a constituent, either because of its low concentration or because it is masked by some trailing contaminant or by heavy concentration of a neighbouring constituent, it is indicated by '?'.

^b Except where indicated below, all material was collected from labelled plants growing in the National Botanic Gardens, Kirstenbosch, Cape (NBG-K).

^c This species has been examined by Van Oudtshoorn.

^d This species belongs to the Grevilleoideae.

^e Herbarium collection of J. P. Rourke, JPR 920.

^f Herbarium collection, NBG-K 87352.

^g This species has been examined by Bate-Smith (1962).

^h Herbarium collection, NBG-K 1768.

ⁱ Herbarium collection, NBG-K 1445.

^j Herbarium collection, NBG-K 85734.

^k Herbarium collection, JPR 25.

these compounds were reported to be absent. In *Mimetes* the former *M. lyrigera* was reported to give a doubtful positive leucoanthocyanin reaction with cyanidin absent. For *M. cucullatus* (= *M. lyrigera*) we obtained a strong leucoanthocyanin reaction and observed cyanidin in the hydrolysate. There also appears to be some confusion over *Protea laurifolia* (= *P. marginata*). *P. laurifolia* is reported to give positive tests for both leucocyanidin and leucodelphinidin whereas *P. marginata* gave a doubtful test for the latter and no leucocyanidin. We obtained cyanidin but no delphinidin from fresh leaves of *P. laurifolia*. We have also obtained cyanidin but not delphinidin from a preserved specimen of *P. simplex*. The same herbarium specimen was previously reported to give both these anthocyanidins.

A general survey of the results in Table I leads to some interesting conclusions:

(1) Except in the case of the rare species, *Orothamnus zeyheri*, leucodelphinidin is always present when myricetin occurs. The leuco-compound **2** however frequently occurs without myricetin. A close study of the results of Bate-Smith (1962) appears to support this observation with but few exceptions. If one accepts the hypothesis that delphinidin and myricetin are formed in the plant from the same 3', 4', 5'-trihydroxy-precursor (Harborne, 1962), one is led to conclude that leucodelphinidin is possibly the precursor of myricetin.

It should be stressed at this point that the absence of leucodelphinidin in the leaf does not necessarily mean its total absence from the plant. For example we have observed delphinidin to be present in the hydrolysate of the bracts of *Protea macrocephala* but the leaves gave only a moderate leucoanthocyanin reaction while the presence of both cyanidin and delphinidin in the hydrolysate was questionable. It would appear therefore that genetic control in the synthesis of flavonoids in the leaf is independent of that in the bracts.

(2) Leucodelphinidin is peculiar not only to the genera *Leucospermum* and *Leucadendron* as observed by Van Oudtshoorn but is also found in the minor genera *Aulax*, *Paranomus*, *Serruria*, *Sorocephalus* and *Spatalla*. Since leucoanthocyanins condense to form tannins (Bate-Smith and Metcalfe, 1957), it is not surprising that the barks of many *Leucospermum* and *Leucadendron* spp were once widely used in tanning (Watt and Breyer-Brandwijk, 1962).

(3) Most *Protea* spp give either weak or negative leucoanthocyanin reactions and myricetin was absent in all forty-three species examined.

(4) Of the flavonoids considered, there appears to be little or no correlation between the presence or absence of kaempferol and the other flavonoids.

TABLE II.

GROUP I:	L-A (+++); M (+); D (+); C (±); Q (+); K (±).
<i>Leucadendron</i> :	<i>L. cinerum</i> ^a ; <i>L. corymbosum</i> ^a ; <i>L. cryptocephalum</i> ; <i>L. discolor</i> ; <i>L. eucalyptifolium</i> ^a ; <i>L. galpinii</i> ; <i>L. gandogerii</i> (= <i>L. guthrieae</i>); <i>L. glabrum</i> ^a ; <i>L. laureolum</i> ; <i>L. rubrum</i> ; <i>L. salicifolium</i> ; <i>L. salignum</i> Berg (= <i>L. adscendens</i> ^a); <i>L. sessile</i> ; <i>L. stellare</i> ; <i>L. tinctum</i> ; <i>L. uliginosum</i> ; <i>L. xanthoconus</i> (= <i>L. salignum</i> R. Br. ^a)
<i>Leucospermum</i> :	<i>L. bolusii</i> ; <i>L. buxifolium</i> ^a ; <i>L. catherinae</i> ; <i>L. cordifolium</i> ; <i>L. patersonii</i> ; <i>L. prostratum</i> ; <i>L. reflexum</i> ; <i>L. rodolentum</i> ; <i>L. tomentosum</i> ^a ; <i>L. truncatulum</i> .
<i>Orothamnus</i> :	<i>O. zeyheri</i> ^b .
<i>Serruria</i> :	<i>S. adscendens</i> ; <i>S. aemula</i> ^c ; <i>S. barbigera</i> ; <i>S. florida</i> .
<i>Spatalla</i> :	<i>S. incurva</i> .
GROUP II:	L-A (+++); M (—); D (+); C (+); Q (+); K (—).
<i>Aulax</i> :	<i>A. cancellata</i> (= <i>A. pinifolia</i>); <i>A. pallasia</i> ^a ; <i>A. umbellata</i> (= <i>A. cneorifolia</i> ^a)
<i>Leucadendron</i> :	<i>L. argenteum</i> ; <i>L. conicum</i> (= <i>L. ramosissimum</i>); <i>L. globularia</i> ; <i>L. lanigerum</i> ; <i>L. longmorensis</i> ; <i>L. modestum</i> ; <i>L. sabulosum</i> ^c ; <i>L. tortum</i> .
<i>Leucospermum</i> :	<i>L. conocarpodendron</i> ^c ; <i>L. cuneiforme</i> ; (= <i>L. attentuatum</i> ^{a,c}) <i>L. lineare</i> ; <i>L. muirii</i> ; <i>L. tottum</i> ; <i>L. vestitum</i> .
<i>Paranomus</i> :	<i>P. medius</i> ^a ; <i>P. reflexus</i> ; <i>P. spicatus</i> .
<i>Protea</i> :	<i>P. welwitschii</i> (= <i>P. hirta</i> ^a)
<i>Serruria</i> :	<i>S. pedunculata</i> (= <i>S. artemesiaefolia</i>).
<i>Sorocephalus</i> :	<i>S. lanatus</i> .
<i>Spatalla</i> :	<i>S. curvifolia</i> .
GROUP III:	L-A (+++); M (—); D (—); C (+); Q (+); K (±).
<i>Diatella</i> :	<i>D. bryiflora</i> .
<i>Leucadendron</i> :	<i>L. abietinum</i> ^a ; <i>L. aemulum</i> ^a ; <i>L. album</i> ; <i>L. crassifolium</i> ^a ; <i>L. daphnoides</i> ; <i>L. loranthifolium</i> ; <i>L. meyerianum</i> ; <i>L. muirii</i> ; <i>L. nobile</i> .
<i>Mimetes</i> :	<i>M. cucullatus</i> (= <i>M. lyrigera</i> ^a); <i>M. fimbriaefolius</i> ; <i>M. hottentoticus</i> .
<i>Protea</i> :	<i>P. barbigera</i> ; <i>P. compacta</i> ; <i>P. harmeri</i> ; <i>P. laurifolia</i> (= <i>P. marginata</i> ^a); <i>P. lorifolia</i> ; <i>P. nerifolia</i> ^c ; <i>P. pityphylla</i> ; <i>P. restionifolia</i> ; <i>P. rupPELLIAE</i> ; <i>P. scabra</i> ; <i>P. simplex</i> ; <i>P. spectosa</i> ; <i>P. witzbergiana</i> .
GROUP IV:	L-A (+++ to —); M (—); D (—); C (—); Q (±); K (±).
<i>Fauvea</i> :	<i>F. macnaughtonii</i> .
<i>Protea</i> :	<i>P. acaulis</i> ; <i>P. acerosa</i> ; <i>P. amplexicaulis</i> ; <i>P. angustata</i> ; <i>P. arborea</i> ; <i>P. aristata</i> ; <i>P. canaliculata</i> ; <i>P. cedromontana</i> ; <i>P. cordata</i> ; <i>P. cynaroides</i> ; <i>P. decurrens</i> ; <i>P. eximia</i> ; <i>P. glabra</i> ; <i>P. grandiceps</i> ; <i>P. lanceolata</i> ; <i>P. longiflora</i> ; <i>P. longifolia</i> ; <i>P. macrocephala</i> (= <i>P. incompta</i> ^a); <i>P. minor</i> ; <i>P. mundii</i> ; <i>P. nana</i> ; <i>P. obtusifolia</i> ; <i>P. odorata</i> ; <i>P. pulchra</i> ; <i>P. repens</i> ; <i>P. rubropilosa</i> ; <i>P. rupicola</i> ; <i>P. scolymocephala</i> ; <i>P. stokoei</i> ; <i>P. sulphurea</i> ; <i>P. venusta</i> .

Footnotes to Table II.

^a Result from Van Oudtshoorn's paper.

Delphinidin absent.

^c Quercetin ? or —.

DISCUSSION

In Table II an attempt to correlate the data presented in Table I has been made. Consequential to the Bate-Smith hypothesis, a grouping of the genera of the Proteoideae based on the flavonoids present was first considered. However, since it was apparent that within each genus specific variations do occur, it was

thought to be more appropriate to group the different species as shown in Table II. Four groups in increasing order of evolutionary advancement become apparent. In order to add weight to the significance of the correlations, the results of Bate-Smith (1962) and Van Oudtshoorn (1963) have been included in the groups.

GROUP I: This comprises those species giving an intense leucoanthocyanin reaction and contain both leucodelphinidin and myricetin. They may thus be regarded as the most primitive species of the Proteoideae living. Leucocyanidin is either absent or only present in low concentration. Quercetin is present in all but one species. The group is dominated by 17 *Leucadendron* spp and 10 *Leucospermum* spp. Four of the five *Serruria* spp examined and one *Spatalla* also belong. Although *Orothamnus zeyheri* gave a negative leucodelphinidin reaction, it has been included with this group as it contains myricetin.

GROUP II: The species here again all give intense leucoanthocyanin reaction but myricetin was not detected. They are therefore more advanced than those in Group I. Leucocyanidin is present in amounts approximately equal to leucodelphinidin and quercetin is present in all but four of the species examined. Eight of the twelve genera examined are represented in this group which is dominated by 8 *Leucadendron* spp and 6 *Leucospermum* spp. The 3 *Aulax* spp and 3 *Paranomus* spp examined all belong to this group as well as one species each of *Serruria*, *Sorocephalus* and *Spatalla*. It is interesting to observe that of the 45 species of *Protea* examined, only *P. welwitschii* falls into this group.

GROUP III: Those species giving a moderate to strong leucoanthocyanin reaction and containing leucocyanidin but neither myricetin nor leucodelphinidin are assigned to this group. Quercetin was present in all but one species. Group III is dominated by 9 *Leucadendron* spp and 13 *Protea* spp. The three *Mimetes* spp examined as well as *Diastella bryiflora* all fall into this group.

GROUP IV: The species in this group gave only a weak to negative leucoanthocyanin reaction. Both leucocyanidin and leucodelphinidin were either absent or, if present, in a concentration too low for detection. Quercetin may or may not be present. The group is largely dominated by 31 *Protea* spp with *Faurea macnaughtonii*, the only other species belonging.

Brabeium stellatifolium, the only indigenous species of the Grevilleoideae, was also examined. It may be described as a Group IV type.

The significance of the data as correlated in Table II becomes clearer when summarised as in Table III. Potential evolutionary trends become apparent as determined solely by the flavonoid constituents and it would appear that the evolutionary line within a given genus does follow the sequence as suggested earlier, thus:

GROUP I → GROUP II → GROUP III → GROUP IV.

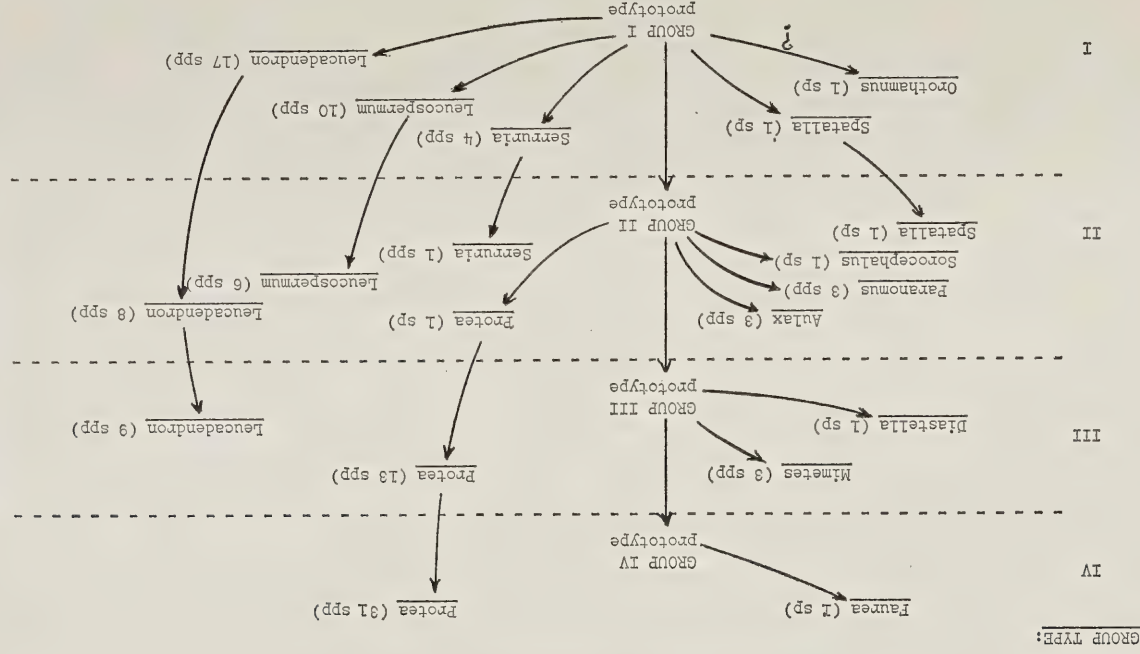
TABLE III

	GROUP I	GROUP II	GROUP III	GROUP IV	No. of Species
L-A	+++	+++	+++	++ to —	
M	+	—	—	—	
D	+	+	—	—	
C	±	+	+	—	
Q	+	+	+	±	
K	±	±	±	±	
GENUS:					
<i>Aulax</i>	0	3	0	0	3
<i>Diastella</i>	0	0	1	0	1
<i>Faurea</i>	0	0	0	1	1
<i>Leucadendron</i>	17	8	9	0	34
<i>Leucospermum</i>	10	6	0	0	16
<i>Mimetes</i>	0	0	3	0	3
<i>Orothamnus</i>	1	0	0	0	1
<i>Paranomus</i>	0	3	0	0	3
<i>Protea</i>	0	1	13	31	45
<i>Serruria</i>	4	1	0	0	5
<i>Sorocephalus</i>	0	1	0	0	1
<i>Spatalla</i>	1	1	0	0	2

Consequently when a Group I species loses genetic control over the oxidation of leucodelphinidin (or the appropriate trihydroxy precursor) to myricetin it becomes a Group II species. Similarly a Group II species on losing genetic control over the synthesis of leucodelphinidin becomes a Group III species, and this in turn becomes a Group IV species when it fails to synthesise leucocyanidin. These deductions indicate that in the leaf the origins of the flavonols myricetin and quercetin are not necessarily parallel as suggested by Harborne (1962). The evidence from our studies strongly suggests that leucodelphinidin is the precursor of myricetin but leucocyanidin cannot be the precursor of quercetin since this flavonol is often found when this leuco-compound is absent.

A possible mode of the evolution of the Proteoideae as determined by the presence or absence of chemotaxonomically significant flavonoids is presented in the figure. It is suggested that the earliest species within a genus arose from a prototype having essentially the same leaf flavonoids. Clearly one prototype can, by losing genetic control over the synthesis of one of these flavonoids, give rise to a more advanced prototype. For clarity each prototype and those species belonging to the same flavonoid group have been separated from dissimilar ones by horizontal lines.

Since only about one quarter of all the Proteoideae have been examined, the scheme can only be regarded as being very tentative at this stage. For a more precise assessment of the prototype giving rise to a particular genus, the whole field of the Proteoideae requires study. One may then find that the smaller



species which have apparently arisen from the prototypes of Group II and III have their origin in the Group I prototype. This may be the explanation of the apparent disparity between *Orothamnus* and *Mimetes*, two morphologically related genera (Johnson and Briggs, 1962). It is therefore necessary to study other *Mimetes* spp to determine possible earlier origins. There is also the possibility that a more primitive species of a genus has become extinct, a hypothesis which cannot be tested but nevertheless cannot be excluded. Such a possibility may apply not only to the genus *Mimetes* but to all the other genera studied. Were this in fact to be true, no definite conclusions can be drawn as to common origins of present living genera, as based solely on the presence or absence of the significant leaf flavonoids. However, the method of testing for these leaf flavonoids is not without merit for it clearly demonstrates the degree of evolutionary development within a given genus. For example it would appear, from present results, that *Serruria* and *Leucospermum* have not advanced as far as *Leucadendron* while *Protea*, arising apparently from a Group II prototype, has advanced most of all.

In concluding this discussion, the results pose a number of interesting questions:

- (1) How do leaf flavonoids change as a result of hybridisation, particularly when species of different groups are crossed?
- (2) Does a change in the leaf flavonoids in a given species indicate a new species or a subspecies? The answer to this question may lead to a remodelling of the larger genera, particularly *Protea* as delimited at present in "Flora Capensis" (Thiselton-Dyer *et al.*, 1912).

The urge to discover the answers to these and other questions justifies extending this survey to include as many as possible, if not all, of the Proteoideae.

ACKNOWLEDGMENTS

The authors sincerely thank Professor H. B. Rycroft, Director of the National Botanic Gardens and his staff for providing material, and Dr. J. P. Rourke, of the Herbarium, Kirstenbosch, for stimulating discussions and assistance. The Council for Scientific and Industrial Research and the University of Cape Town are thanked for grants.

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